Antimicrobial and antioxidant screening of N′-substituted sulphonyl and benzoyl derivatives of 4-Pyridine carboxylic acid hydrazide

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Abstract: In this research program, the antibacterial, antifungal and antioxidant activities of six N□-substituted sulfonyl and benzoyl derivatives of lead molecule PCH were reported. Out of these compounds, sulphonyl derivatives **2**,**3** and benzoyl derivative **5** showed moderate to good activity against different strains of gram-positive and gram-negative bacteria including *B. cereus*, *B. subtilis*, *B. thruingiensis* and *S. pyogenes*, *S. fecalis* and *E. coli* ATCC 8739. Moreover, upon antifungal screening, the compound, N′-[(2,4,6-trimethylbenzene) sulfonyl]pyridine-4-carbohydrazide possessed good antifungal activity against Candida species, a causative agent of systemic fungal infections. Antioxidant study demonstrated more than 50% inhibition in DPPH assay for sulphonyl derivative 2 indicating its potential as antioxidant while the other derivatives expressed low level of radical scavenging property.

Keywords: Pyridine carboxylic acid hydrazide, antimicrobial activity, antioxidant activity, DPPH assay.

INTRODUCTION

A lot of antimicrobial and antioxidant agents are available in the market. Even then there is always a need of new molecules to combat the diseases associated with pathogenic microbes and free radicals. The reason is the problem with these antimicrobial drugs, which become resistant against the microorganisms after certain use making them infective and limit their application. Moreover, the resistance is also produced due to the easily available OTC antibiotics, which are self-medicated improperly without knowing the stated dose and duration hence becoming a major cause of morbidity and mortality (Ghannoum and Rice, 1999).

Medicinal chemists make efforts to produce agents with more than one medical application. Keeping this in view, we synthesized six new derivatives of PCH. The synthetic procedures and their characterization have been reported in detailed earlier (Naeem *et al.*, 2014), which are summarized briefly in table 1.

PCH (4-Pyridine carboxylic acid hydrazide) is known for its anti-tubercular activity and currently in use to cure tuberculosis under the name of Isoniazid. Literature revealed the importance of PCH derivatives to possess antimicrobial (Sankar and Pandiarajan, 2010; Malhotra *et al*., 2012a), antioxidant (Matei *et al.*, 2013; Malhotra *et al.*, 2012b) and central nervous system activities (Joshi, 1976; Carta *et al.*, 2008). Therefore, we tested these new derivatives to evaluate their potential as antimicrobial and antioxidant agents.

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MATERIAL AND METHODS

In vitro screening of antibacterial activity

The pathogenic bacteria were obtained from the Department of Microbiology, Federal Urdu University of Arts, Science and Technology, Karachi-Pakistan. The antibacterial determination of all the six derivatives was performed against eleven gram-positive and seventeen gram-negative species of bacteria using Agar-well method. All the bacterial isolates were tested for identity and purity and kept on nutrient agar at 4°C in the refrigerator till use. Autoclaved Muller Hinton broth was used to maintain the bacterial culture; afterwards wells were dug onto Muller Hinton Agar and then 10µL of culture were poured into the wells and loaded with the test samples (Perez *et al.*, 1990). Gentamicin antibiotic was served as control for the study (Vaghasiya *et al.*, 2009). After incubating the plates at 28±2°C for 24-48hours, diameter of zone of inhibition was measured using vernier caliper.

In vitro screening of antifungal activity

The test organisms were obtained from the Department of Microbiology, Federal Urdu University of Arts, Science and Technology, Karachi-Pakistan and included members of the 6 saprophytic fungi5 dermatophytes and 6 yeast species. All the fungal isolates were checked for purity and maintained on Sabourd Dextrose agar (SDA) (Oxoid, Basingstoke-UK) at 4°C in the refrigerator until use. Antifungal activity of the parent and synthesized molecules was determined by agar-well method. The fungal spore suspension was prepared in autoclaved distilled water and transferred aseptically into each SDA

plates (Wuthi-udomlert and Vallisuta, 2011). All the plates were incubated at 28±2°C for 24-48hours and after incubation diameter of zone of inhibition was measured by verniercaliper. Griseofulvin, antifungal agent was used as a control.

Determination of minimum inhibitory concentration (MIC)

MIC of the test compounds was carried out by Micro broth dilution method using 96-well microtitre plate (Sherwani *et al.*, 2012). Standard solution of 100mg/mL of test compounds were prepared in distilled water. Two fold serial dilutions was made in 100µL broth and then 10µL of two hours old culture matched with 0.5 Mac Farland index later was added in all wells. One well served as antibiotic control while other served as culture control. Micro titre plate was incubated for 24hours at 37°C. The MIC was read as the well showing no visible growth.

In-vitro screening of antioxidant activity

Determination of antioxidant activity of the synthesized derivatives was carried out by method described by Lee *et al*. (Lee *et al.*, 1998). 1,1-diphenyl-2-picrylhydrazyl (DPPH) was prepared in ethanol (300µM). Briefly, 10µL of test sample and 90µL solution of stable radical, DPPH was added in 96-well micro titer plates and incubated at 37°C for 30minutes. Absorbance was measured at 515nm by using a spectrophotometer. Percent inhibition of radicals by treatment of test sample was determined by comparison with a DMSO treated control group.

$$\% \text{ Inhibition} = \frac{\text{absorbance of the control} - \text{absorbance of the test sample}}{\text{Absorbance of the control}} \times 100$$

Ascorbic acid was used as standard control. The EC_{50} value calculated denotes the concentration (in µg/ml) of sample required to scavenge 50% of DPPH.

RESULTS

The results of *in-vitro* antibacterial and antifungal assessments are presented in table 2 and 3 in terms of zone of inhibition (mm) respectively. The observed antimicrobial MIC values are tabulated in tables 4 and 5. The antioxidant activity of the compounds is shown in fig. 1 in terms of percent inhibition.

As shown in table 2, compounds 1, 4 and 6 did not produce any activity against tested bacterial strains. When compared to PCH, derivative2 possessed good antibacterial activity against gram-positive species such as *B. cereus, B. subtilis, B. thruingiensis* and *S. pyogenes* while Compounds 3 and 5 showed mild to moderate activity for the same. Compounds 3 and 5 were found moderately active against gram-positive *S. epidermidis* whereas derivative 3 exhibited good potential against *S. saprophyticus, S. fecalis.*

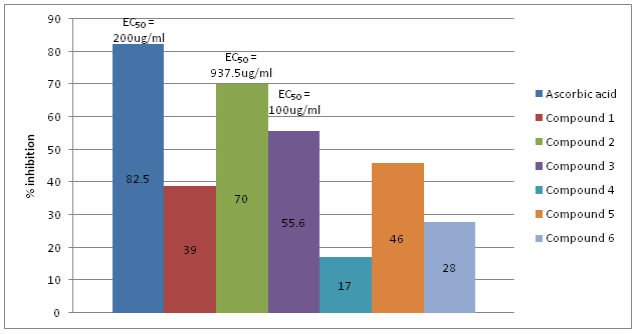


Fig. 1: *In vitro* Antioxidant activity of the test compounds 1-6

Among gram-negative species, compound 2 showed moderate activity against *Enterobacteraerogenes, E. coli ATCC8739, E. coli* and *E. coli MDR*. Compound 3 displayed good activity against *Klebsiella pneumonia*.

Form table 3, it can be observed that the synthesized derivatives, 2and 3were succeeded to attainpotent activity against *C. albicans*, *C. albiacans ATCC 0383*, *C. galbrata*, *C. tropicalis* and *Rhizopus* as compared to PCH and Griseofulvin while compounds 1, 4, 5 and 6 did not display antifungal activity against the tested strains of yeast, dermatophytes and saprophytes. The parent molecule, PCH and its two products (2 and 3) were inactive against dermatophytes but they showed potential against Candida and Rhizpopus species.

DISCUSSION

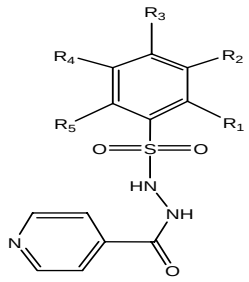
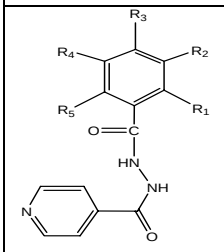
Antimicrobial activity

By looking in to the structures of these derivatives, the presence of three methyl groups at *ortho-para* positions in compound 2made this molecule more active as compare to its parent PCH against different strains of bacteria and fungi. Interestingly, one methyl group at *para* position in compound 1 was responsible to attenuate antimicrobial effects of PCH. Therefore, it is suggested that the *ortho-para* substitution is somewhere important for antimicrobial activity. The result is supported by the findings of Judge *et al.*, 2013 who explored that the derivatives with *ortho* substituents displayed more antimicrobial effects.

The observed antimicrobial activity in compound 3 may be attributed to the attachment of electron with drawing bromo group at *para* position which resulted in the increment of antibacterial potency of the synthesized compound (Judge *et al.*, 2012).

It was observed that the active compound (5) in benzoyl derivatives (5 and 6) has shown potential for antibacterial effect, indicating that substitution of methyl group at *para*

Table 1: Physical data of synthesized compounds 1-6

Structural Representation	Compounds	R_1	R_2	R_3	R_4	R_5	MP (°C)	Molecular Formula	IR (cm^{-1})	^{1}H-NMR (δ)
 N□-substituted arylsulphonyl pyridine-4-carbohydrazide	1	H	H	$-CH_3$	H	H	190-194	$C_{13}H_{13}N_3O_3S$	3117 (NH), 2971, 2811 (Ar-CH_3)	11.88 (s, NH), 2.04-2.07 (d, N′, CH_3)
	2	$-CH_3$	H	$-CH_3$	H	$-CH_3$	207-210	$C_{15}H_{17}N_3O_3S$	3111 (NH), 2967, 2814 (Ar-CH_3)	11.09 (s, NH), 2.27 (s, CH_3), 1.80 (s, N′)
	3	H	H	-Br	H	H	217-222	$C_{12}H_{10}BrN_3O_3S$	3104 (NH)	11.08 (s, NH), 1.80 (s, N′)
	4	H	H	$-NO_2$	H	H	193-197	$C_{12}H_{10}N_4O_5S$	3184 (NH), 1525, 1354 (Ar-NO_2)	11.88 (s, NH), 1.94 (s, N′)
 N□-substituted benzoyl-pyridine-4-carbohydrazide	5	H	H	$-CH_3$	H	H	227-231	$C_{14}H_{13}N_3O_2$	3093 (NH), 2969, 2637 (Ar-CH_3)	11.52 (s, NH), 11.63 (s, N′)
	6	H	$-NO_2$	H	$-NO_2$	H	225-228	$C_{13}H_9N_5O_6$	3093 (NH), 1538, 1351 (Ar-NO_2)	11.35 (s, NH), 11.53 (s, N′)

position on benzoyl ring contributes the antibacterial activity. Whereas the same substitution in sulphonyl ring completely demolished the antibacterial effects.

Regarding antifungal activity of these derivatives, compounds 2 and 3 exhibited more zone of inhibition against Candida species as compare to parent and standard hence the antifungal results are considered to be potent and encouraging. These active compounds contain methyl and bromo groups. Therefore it is suggested that these two groups ($-CH_3$ and -Br) in connection with sulphonyl moiety when attached to PCH would behave as antimicrobial agents.

MIC is the minimum concentration of an antimicrobial that will inhibit the visible growth of organism after overnight incubation (Andrews, 2001). The MIC (in mg/ml) values in table 4 showed that the active derivatives inhibit bacterial growth at much less MIC values than PCH, indicating that these derivatives are more potent than the parent molecule.

Antioxidant activity

DPPH is one of the stable free radicals with deep purple color and is used to determine the free radical scavenging abilities. The technique is based on the principle of changing the color of DPPH from purple to yellow when it is reduced by chemicals having antioxidant property (Lu *et al.*, 2010; Kedare and Singh, 2011).

The $EC_{50}$of the antioxidants is the concentration that causes a decline in the initial DPPH-radical concentration to 50% (Prior *et al.*, 2005) and is the indicator to guage the extent of antioxidant property of the compounds in terms of %RSA (Radical Scavenging Activity).

From fig. 1, it can be noticed that the sulphonyl compound 2 with three methyl groups at *ortho, para positions* and compound 3 with *para* bromo attachment were effective in inhibiting the DPPH concentration up to 70% and 55.6% with EC_{50} values 937.7 and 100µg/ml respectively.

Table 2: *In vitro* antibacterial activity of PCH and test compounds 1-6

Compounds	Zone of inhibition in mm							
Bacteria	Gentamicin	PCH	01	02	03	04	05	06
Gram Positive Bacteria								
Bacillus cereus	22	08	_	17	10	_	10	_
Bacillus subtilis	23	10	_	18	15	_	16	_
Bacillus thruingiensis	15	10	_	16	14	_	13	_
Corynebacteriumdiptheriae	-	_	_	_	_	_	_	_
Corynebacteriumhofmanii	-	_	_	_	_	_	_	_
Corynebacteriumxerosis	-	_	_	_	_	_	_	_
M. smegmatis	-	_	_	_	_	_	_	_
Staphylococcus epidermidis	13	08	_	_	12	_	13	_
Streptococcus fecalis	13	_	_	_	16	_	_	_
Streptococcus pyogenes	20	13	_	21	13	_	13	_
Streptococcus saprophyticus	19	_	_	_	17	_	_	_
Gram Negative Bacteria								
Acinetobacterbaumanii	-	16	_	13	18	_	14	_
Aeromonashydrophila	-	19	_	_	_	_	17	_
Campylobacter coli	-	_	_	_	_	_	_	_
Campylobacter jejuni	-	_	_	_	_	_	_	_
E. coli multi drug resistance	21	_	_	14	_	_	_	_
Enterobacteraerogenes	19	_	_	12	_	_	_	_
Escherichia coli	25	_	_	12	_	_	_	_
Escherichia coli ATCC 8739	20	07	_	15	_	_	_	_
Helicobacter pylori	-	_	_	_	_	_	_	_
Hemophilus influenza	-	_	_	_	_	_	_	_
Klebsiella pneumonia	13	_	_	_	18	_	_	_
Salmonella paratyphi A	13	_	_	_	_	_	_	_
Salmonella paratyphi B	13	_	_	_	_	_	_	_
Salmonella typhi	16	_	_	_	_	_	_	_
Serratiamarcesens	-	_	_	_	_	_	_	_
Shigelladysenteriae	08	_	_	_	_	_	_	_
Vibrio cholera	-	_	_	_	_	_	_	_

Table 3: *In vitro* antifungal activity of PCH and the test compounds 1-6

Compounds	Zone of inhibition in mm							
Fungi	Griseofulvin	PCH	01	02	03	04	05	06
Yeast								
Candida albicans	04	13	_	18	14	_	_	_
Candida albicans ATCC 0383	06	08	_	15	12	_	_	_
Candida galbrata	05	13	_	19	17	_	_	_
Candida kruzei	04	13	_	12	15	_	_	_
Candida tropicalis	05	12	_	17	10	_	_	_
Saccharomyces cerevisiae	-		_	_	_	_	_	_
Dermatophytes								
Microsporumcanis	-	_	_	_	_	_	_	_
Microsporumgypseum	-	_	_	_	_	_	_	_
Trichophytonmentagrophytes	-	_	_	_	_	_	_	_
Trichophytonrubrum	-	_	_	_	_	_	_	_
Trichophytontonsurans	-	_	_	_	_	_	_	_
Saprophytes								
Aspergillusflavus	04	13	_	_	_	_	_	_
Aspergillusniger	04		_	_	_	_	_	_
Fusarium specie	-		_	_	_	_	_	_
Helminthosporum	-		_	_	_	_	_	_
Penicilliumsp	-		_	_	_	_	_	_
Rhizopus	-	10	_	16	10	_	_	_

(-) indicates no activity, ≤10=mild, 11–15 = moderate and Above 15=good

Table 4: Antibacterial minimum inhibitory concentration values of compounds 2, 3, and 5

Compounds	MIC (in mg/ml)			
Bacteria	PCH	02	03	05
Gram-positive bacteria				
Bacillus cereus	120	80	80	88
Bacillus subtilis	190	80	44	74
Bacillus thruingiensis	210	74	60	80
Staphylococcus epidermidis	184	-	22	12
Streptococcus fecalis	-	-	12	-
Streptococcus pyogenes	100	22	34	22
Streptococcus saprophyticus	-	-	10	-
Gram-negative bacteria				
Acinetobacterbaumanii	122	92	74	84
Aeromonashydrophila	100	-	-	82
E. coli multi drug resistance	-	84	-	-
Enterobacteraerogenes	-	38	-	-
Escherichia coli	-	24	-	-
Escherichia coli ATCC 8739	166	44	-	80
Klebsiella pneumonia	-	-	34	-

Table 5: Antifungal minimum inhibitory concentration values of compounds 2 and 3

Compounds	MIC (in mg/ml)		
Fungi	PCH	02	03
Yeast			
Candida albicans	130	180	198
Candida albicans ATCC 0383	210	200	120
Candida galbrata	180	120	144
Candida kruzei	212	158	189
Candida tropicalis	190	156	210
Saprophytes			
Aspergillusflavus	110	_	_
Rhizopus	150	170	200

(-) indicates no activity

Compound 5 having *para* methyl group at benzoyl moiety also revealed mild antioxidant activity with 46% DPPH inhibition. Other synthesized derivatives demonstrated lesser antioxidant effects.

CONCLUSIONS

The end result of this study concluded that the chemical modification of the parent compound PCH with sulphonyl and benzoyl substitutions aids in its antibacterial potency as obvious with less MIC values in compound 2, 3 and 5. The synthesized compounds have also proved as good antifungal agents, which is obvious by more zone of inhibition for active compounds 2 and 3. These encouraging results help us in designing of active and/or potent antimicrobial drug molecules.

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